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Influence of nitric oxide on morphine-induced conditioned place preference in the rat central amygdala

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Abstract

Effects of intra-central amygdala injections of L-arginine, a nitric oxide (NO) precursor, and N^G-nitro-L-arginine methyl ester (L-NAME), a NO synthase (NOS) inhibitor, on morphine-induced conditioned place preference in rats were investigated by using an unbiased 3-day schedule of place conditioning design. Animals receiving once daily injections of morphine (0.5–7.5 mg/kg, subcutaneously, s.c.) or saline (1.0 ml/kg, s.c.) showed a significant place preference in a dose-dependent manner. The maximum response was observed with 5.0 mg/kg of the opioid. Co-administration of morphine (5.0 mg/kg) with L-arginine (0.3, 1.0 and 3.0 μg/rat), but not with L-NAME (0.3, 1.0 and 3.0 μg/rat), during the acquisition of morphine-induced conditioned place preference increased morphine-induced conditioned place preference. The response to L-arginine was blocked by L-NAME preadministration. L-arginine and L-NAME by themselves did not induce conditioned place preference. When L-arginine or L-NAME at 0.3–3.0 μg/rat was administered 1 min before conditioned place preference testing, L-arginine but not L-NAME caused an increase in the expression of morphine-induced conditioned place preference, the effect that was blocked by L-NAME preadministration. A dose of L-arginine (0.3 μg/rat), but not L-NAME, during expression of morphine-induced conditioned place preference produced an increase in locomotion compared with that in the control group. It may be concluded that an increase in the NO levels in the central amygdala may have an effect on the acquisition and expression of morphine-induced conditioned place preference.

Keywords: Morphine; Conditioned place preference; Central amygdala; Nitric oxide (NO); L-arginine; L-NAME (N^G-nitro-L-arginine methyl ester); Rat

1. Introduction

There is evidence that implicates the mesolimbic dopamine pathway in acquisition and expression of the opiate-induced conditioned place preference (Carr and White, 1983, 1986; Wise, 1987), but little is known about the involvement of other limbic structures in this type of learning (Hiroi and White, 1991). However, a consideration of the anatomical connections of the mesolimbic system leads to several obvious possibilities. For example, the central amygdala receives a wide range of ascending thalamic and brainstem inputs including a dopaminergic input from the ventral midbrain (Fallon and Moore, 1978). Neurons originating from the central amygdala strongly contribute to the antinociceptive effect of systemically administered morphine (Manning and Mayer, 1995a,b). After unilateral inactivation of the central amygdala, there is a lateralized deficit in morphine antinociception (Manning, 1998). Stinus et al. (1990) showed that the central amygdala is involved in opiate withdrawal-induced conditioned place aversion. Furthermore, it has been suggested that amygdala activity influences memory by modulating consolidation processes in other brain regions (McGaugh et al., 1993). Other evidence also suggests that the amygdala is critical for emotional conditioning (McGaugh et al., 1992; LeDoux, 1993; Davis, 1997). Lu et al. (2000), using microinjection of specific inhibitors of Ca^{2+}/calmodulin-dependent protein kinase II into the amygdala showed that the amygdala is involved in regulating the dependence and the relapse to morphine. McLntyre et al. (1998) indicated that lesions of the amygdala impair performance in a conditioned place preference. Mice in which a bilateral amygdala lesion was made before or after conditioned individual preference training (a modified conditioned place preference test) did not show any preference for either compartment of the preference box as shown by Borlongan and Watanabe (1994).

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Nitric oxide (NO) is formed enzymatically from L-arginine by NO synthase (NOS) after the activation of the NMDA receptor (Moncada et al., 1991). The NO-forming enzyme is inhibited by several derivatives of L-arginine (Rees et al., 1990; Thorat et al., 1994).

NO is involved in a variety of physiological functions such as vasodilation (Moncada et al., 1991), immune cell regulation (Moncada et al., 1995), neuronal differentiation (Nicotera et al., 1995) and memory (Schuman and Madison, 1991). NO is shown to play a role in the expression (Dambisya and Lee, 1996) and development (Majeed et al., 1994; Machelska et al., 1997; Lue et al., 1999) of morphine tolerance and dependence in laboratory animals. Extensive evidence also indicates NO involvement in the rewarding properties of opiates (Kivastik et al., 1996; Itzhak et al., 1998). A survey of the behavioral effects of N^G-nitro-L-arginine methyl ester (L-NAME), the non-selective inhibitor of NOS, on morphine withdrawal in rats showed that this agent reduced some signs of morphine withdrawal in the rats (Vaupel et al., 1995). L-nitroarginine (L-NOARG), an inhibitor of NOS, when given at 20 mg/kg intraperitoneally, significantly inhibited the morphine-induced place preference in rats (Kivastik et al., 1996).

Since the effects of intra-central amygdala injections of NO agents, L-arginine and/or L-arginine derivatives (e.g. L-NAME), on morphine-induced conditioned place preference have not been determined, the present study attempted to elucidate the effects of such treatments on the acquisition and expression of morphine-induced conditioned place preference in rats.

2. Materials and methods

2.1. Subjects

Subjects were male Wistar rats (Pasteur Institute of Iran, Tehran, Iran) weighing between 255 and 275 g at the start of the experiments. The animals were housed in a controlled colony room (temperature, 22 ± 3 °C) at four per cage. They were maintained on a 12-h-light/dark cycle (0700–1900 h) with food and water ad libitum. They were housed in the colony for at least 1 week prior to commencement of the experiments. Testing was conducted during the light phase. Each animal was used once. The protocol was approved by the Ethics Committee of the Faculty of Science of Tehran University (no. 357; November 8, 2000).

2.2. Drugs

The drugs that were used in the present study were morphine sulfate (Temad, Tehran, Iran), L-arginine (Sigma, St. Louis, MO, USA), N^G-nitro-L-arginine methyl ester (L-NAME; Research Biochemical, Natick, MA, USA) and sodium pentobarbital (Sigma). These drugs were freshly prepared in sterile 0.9% NaCl solution. Morphine and pentobarbital were injected subcutaneously (s.c.) and intraperitoneally, respectively. L-arginine and L-NAME were injected bilaterally into the central nucleus of the amygdala. Vehicle injections were 0.9% physiological saline.

2.3. Surgery

The animals were anesthetized with sodium pentobarbital (45 mg/kg) and placed in a stereotaxic apparatus, with the incisor bar at approximately 3.3 mm below horizontal zero to achieve a flat skull position. An incision was made to expose the skull. Two holes were drilled in the skull at stereotaxic coordinates AP −2.12 mm posterior to bregma, and L ±4.1 mm according to the atlas of Paxinos and Watson (1987). Two guide cannulae (21-gauge) were inserted into the holes. For animals receiving bilateral injections into the central amygdala, the guide cannulae were lowered 7.3 mm below the bregma through the holes drilled at the desired coordinates. The guide cannulae were anchored with a jeweler's screw, and the incision was closed with dental cement. After surgery, dummy inner cannulae that extended 0.5 mm beyond the guide cannulae were inserted into the guide cannulae, and left in place until injections were made. All animals were allowed to recover for 1 week before behavioral testing began.

2.4. Intra-central amygdala injection

The animals were gently restrained by hand; the dummy cannulae were removed from the guide cannulae. For intra-central amygdala injections of drugs, a 5.0-μl glass Hamilton syringe was used. The injection (inner) cannulae (27-gauge), which projected a further 0.5 mm ventral to the tip of the guides, were attached with polyethylene tubing (0.6 mm internal diameter) to the Hamilton syringe. The injection volume was 1.0 μl (0.5 μl per side) for all groups. Injections were made over a 30-s period, and the injection cannulae were left in the guide cannulae for an additional 60 s to facilitate diffusion of the drugs.

2.5. Histological verification

After behavioral testing, the animals were anesthetized with chloroform. They were then injected with 0.5 μl (per side) of 1% methylene blue solution using 27-gauge injection cannulae that projected a further 0.5 mm ventral to the tip of the guides. The brains were removed and placed in 10% formalin solution for 10 days before sectioning. The fixed brains were then sliced directly across the injection sites, and the cannulae placements were verified using the atlas of Paxinos and Watson (1987). The results of histological examination for central amygdala injection cannulae placements are shown in Fig. 1. Only data from rats that received histologically verified injections were included for analyses.

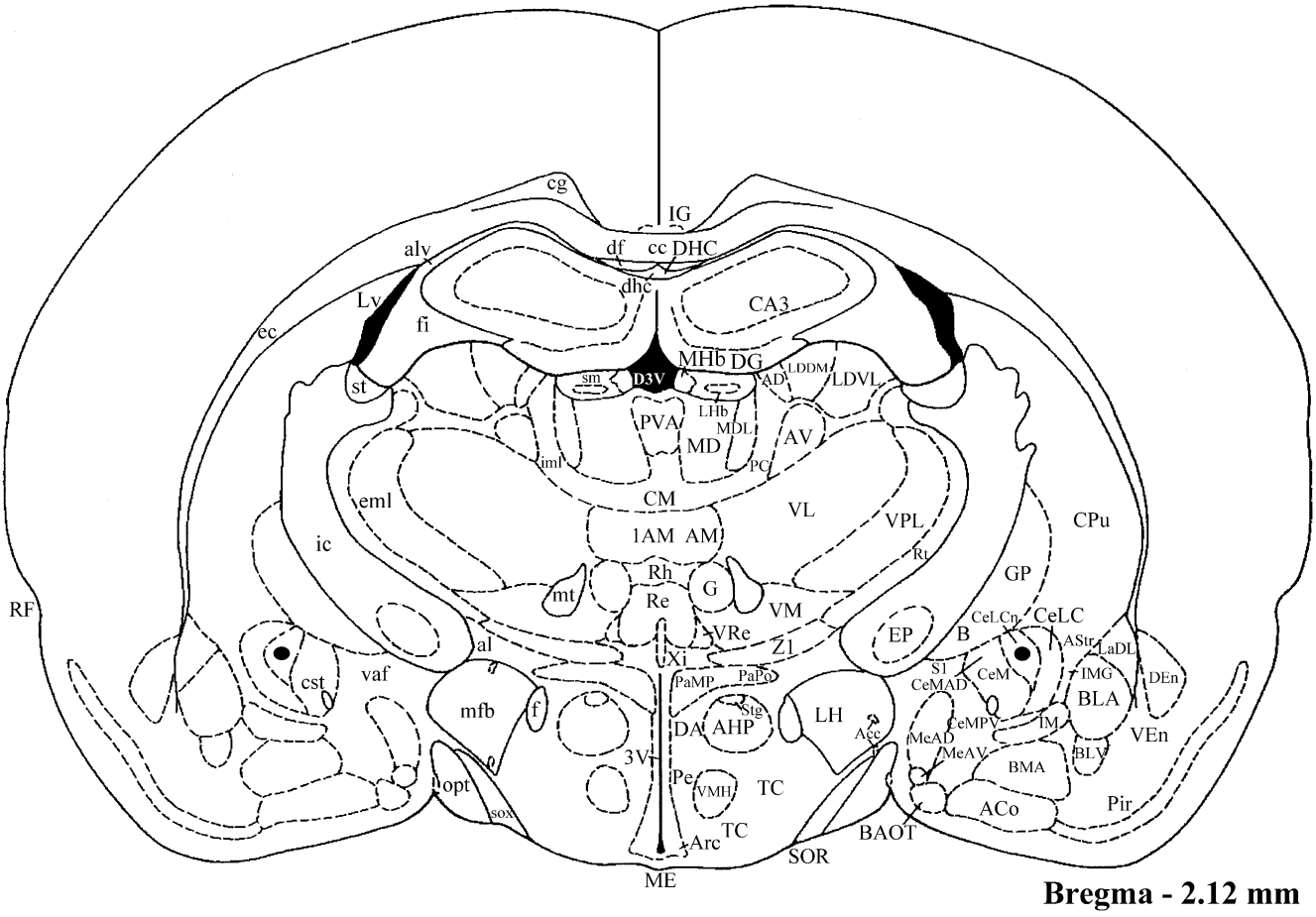

Fig. 1. Cannulae placements in central amygdala for conditioned place preference (AP: −2.12). Verification from atlas of Paxinos and Watson, 1987.

2.6. Apparatus

A two-compartment conditioned place preference apparatus (30 × 60 × 30 cm) was used in these experiments. Place conditioning was conducted using an unbiased procedure, with minor changes to the design previously described (Shippenberg et al., 1996). The apparatus was made of wood. Both compartments were of identical size (the apparatus was divided into two equal-sized compartments by means of a removable white wall) and shading (both were white), but distinguished by texture and olfactory cue. To provide the tactile difference between the compartments, one of the compartments had a smooth floor while the other compartment had a white nylon mesh floor. A drop of vinegar was placed at the right corner of the compartment with a textured (nylon mesh) floor, and a drop of rose perfume was placed at the left corner of the other compartment to provide the olfactory cue difference between compartments. Two compartments also had different black stripes (on three of their sides). In this apparatus, the rats showed no consistent preference for either compartment.

2.7. Procedure

The procedure that was used for place conditioning consisted of the three following phases.

2.7.1. Familiarization or habituation

On day 1, the animals were accustomed to the conditioned place preference apparatus for 15 min. The removable wall was raised, thereby allowing each rat to move freely between the two compartments. Animals were then randomly assigned to one of two groups (four rats in each group) for place conditioning and a total of eight animals were used for all subsequent experiments.

2.7.2. Conditioning or induction phase

Place conditioning or the induction phase started 1 day after habituation. This phase consisted of six, 45-min sessions (three saline and three drug pairing). These sessions were conducted twice each day (from day 2 to day 4) with a 6-h interval. On each of these days, separate groups of animals received one conditioning session with morphine and one with saline. During these sessions, the removable wall was

inserted along the seam separating the two compartments and each group of rats was then confined to one compartment. Animals of each group were injected with morphine and were immediately confined to one compartment of the apparatus for 45 min. Following administration of saline, the animals were confined to the other compartment for 45 min. Treatment compartment and order of presentation of morphine and saline were counterbalanced for either group. Conditioning was conducted as previously described in detail, using an unbiased procedure (Shippenberg et al., 1996).

2.7.3. Testing phase

The test session was carried out on day 5, 1 day after the last conditioning session, in a morphine-free state. Each animal was tested once only. For testing, the removable wall was raised and each uninjected animal (with morphine) was allowed free access to the two compartments of the apparatus for 15 min. An observer then assessed the time spent in the morphine- and saline-paired compartments. The location of the animal was determined by the position of the front paws. The scores (conditioning scores) represent the time spent in the drug-paired compartment minus the time spent in the saline-paired compartment, and are expressed as means ± S.E.M.

2.8. Induction and assessment of morphine place conditioning in opioid-naive rats

In a pilot study, the effects of subcutaneous (s.c.) administration of various morphine doses (0.5, 1.25, 2.5, 5.0 and 7.5 mg/kg) on the induction of conditioned place preference were determined. Morphine or saline was injected in a 3-day schedule of conditioning as described more in detail in Materials and methods. Conditioned place preference induction was assessed by determining the time spent in the morphine- and saline-paired compartments of the conditioned place preference apparatus during 15-min testing of either group in a morphine-free state. To examine state-dependent learning (as mentioned by Overton, 1973), morphine was administered prior to testing. It was found that the size of the conditioned place preference response was not altered. Therefore, the animals were tested in a morphine-free state. This eliminates the possibility that morphine-induced motor effects influence responding (Olmstead and Franklin, 1997b).

2.9. Measurement of effects of L-arginine (NO precursor) and L-NAME (NOS inhibitor) on the acquisition and expression of morphine-induced conditioned place preference

The effects of either L-arginine or L-NAME on the acquisition and expression of morphine-induced conditioned place preference in rats were determined in the two procedures as follows.

1. Conditioning was conducted as described above for the conditioning phase. The opioid-naive animals received L-arginine or L-NAME along with morphine during the conditioning phase to determine the effects of the drugs on the acquisition of morphine-induced conditioned place preference. L-arginine or L-NAME at 0.3–3.0 μg/rat was injected once/daily, for 3 days, 1 min before the administration of morphine (three-pairing). The respective control groups received saline in a volume of 0.5 μl per side, intra-central amygdala, three-pairing. All conditioning sessions (six sessions) were 45 min in duration.
2. Animals were conditioned with morphine as described in the place conditioning procedure. In order to test the effects of L-arginine or L-NAME on the expression of morphine-induced conditioned place preference, the drugs, 0.3–3.0 μg/rat, were injected once on the day of testing (day 5), 1 min prior to the conditioned place preference testing. The respective control groups received saline in a volume of 0.5 μl per side, intra-central amygdala, pre-testing.

To determine the probable reversal effect of L-NAME on the response induced by L-arginine, L-NAME at 0.3–3.0 μg/rat was administered intra-central amygdala, 1 min prior to the administration of the effective dose of L-arginine in both experiments.

2.10. Effects of L-arginine and L-NAME, alone or in combination, on locomotor activity

Locomotion was determined after intra-central amygdala injections of either L-arginine (0.3, 1.0 and 3.0 μg/rat) or L-NAME (0.3, 1.0 and 3.0 μg/rat), alone or in combination, in the place conditioning apparatus. To measure locomotor activity, the ground area of the conditioned place preference compartments were divided into four equal-sized squares. Locomotion was thus measured as crossing from one square to another. The results are expressed as counts per animal over a 15-min testing period after drug administration, on the drug-paired side. Control groups received saline.

2.11. Statistical analysis

Two-way analysis of variance (ANOVA) or, when appropriate, one-way ANOVA followed by the Tukey–Kramer or Newman Keul's multiple comparison tests were used to determine the effects of the various treatments on morphine-induced place conditioning. *P*-values less than 0.05 were considered as significant.

3. Results

3.1. Dose–response curve for place preference conditioning produced by morphine in rats

The animals (four per group; eight in each experiment) received subcutaneous (s.c.) injections of saline or morphine

in a 3-day schedule of conditioning as described in Materials and methods.

Fig. 2A shows the dose–response curve for place conditioning induced by morphine in rats. Animals, which received saline (1.0 ml/kg) twice per day, during six sessions, exhibited no preference for either place cue. The mean time spent in the smooth floor compartment minus that spent in the textured one was 9 s. Administration of different doses of morphine (0.5, 1.25, 2.5, 5.0 and 7.5 mg/kg) during conditioning induced conditioned place preference [one-way ANOVA; $F(5,42)=7.6$, $P<0.0001$]. Morphine 0.5 and 1.25 mg/kg failed to produce significant conditioning in animals and no preference for either place cue was seen. The maximum response was observed with 5.0 mg/kg of the opioid. In view of the results, morphine 5.0 mg/kg during conditioning sessions was used for subsequent studies. Fig. 2B indicates that morphine administration (s.c.) had no significant effect on locomotor activity [one-way ANOVA; $F(5,42)=0.3$, $P>0.05$].

3.2. Effect of NO synthesis precursor and inhibitor on the acquisition of morphine-induced conditioned place preference

Fig. 3A shows the effect of L-arginine, with or without morphine, on conditioned place preference. L-arginine or

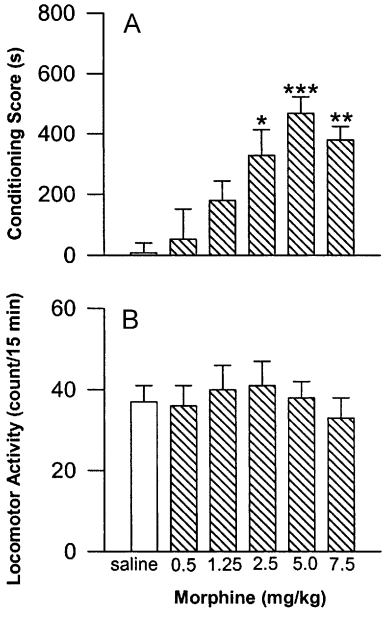

Fig. 2. Dose–response curve for morphine-induced conditioned place preference. The different doses of morphine (0.5–7.5 mg/kg) or saline (1.0 ml/kg) were given subcutaneously (s.c.) in a 3-day schedule of conditioning. The control group received saline (1.0 ml/kg, s.c.) twice daily for 3 days. The data are expressed as mean conditioning scores ± S.E.M. The conditioning score is defined as the time (in s) spent in the drug-paired place minus that spent in the saline-paired place (A). Locomotor activity was assessed as described in Materials and methods (B). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ different from the saline control group.

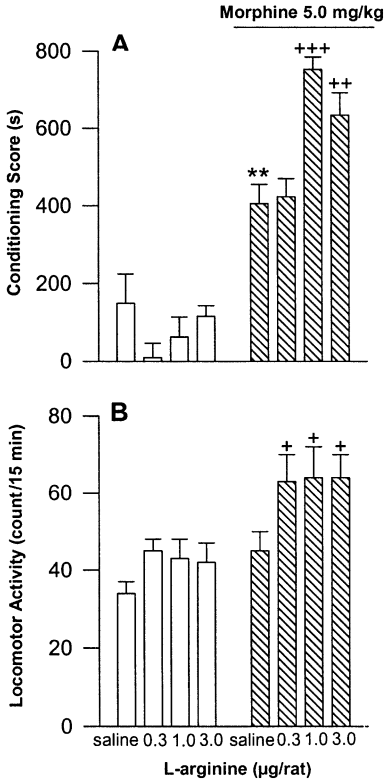

Fig. 3. Effect of L-arginine with or without morphine on conditioned place preference. Animals received saline (1.0 μl/rat, intra-central amygdala) or L-arginine (0.3, 1.0 and 3.0 μg/rat, intra-central amygdala) in the presence or absence of morphine (5.0 mg/kg, s.c.) during conditioning. The data are expressed as mean conditioning scores ± S.E.M. The conditioning score is defined as the time (in s) spent in the drug-paired place minus that spent in the saline-paired place (A). Locomotor activity was assessed as described in Materials and methods (B). ** $P<0.01$ different from the saline control group. + $P<0.05$, ++ $P<0.01$, +++ $P<0.001$ different from the respective morphine control group.

L-NAME was used in combination with morphine during conditioning (as described in Materials and methods). Two-way ANOVA indicates a significant difference between the response to L-arginine (0.3, 1.0 and 3.0 μg/rat) and that to L-arginine plus morphine (5.0 mg/kg) [Factor morphine, $F(1,56)=177.6$, $P<0.0001$; Factor L-arginine, $F(3,56)=6.6$, $P<0.001$; Factor morphine × L-arginine, $F(3,56)=7.0$, $P<0.0001$]. Further analysis showed that morphine, but not L-arginine, induced conditioned place preference and that L-arginine enhanced morphine-induced conditioned place preference.

Fig. 3B shows the response to L-arginine and/or morphine on locomotion. Two-way ANOVA indicates no interaction between the response to L-arginine (0.3, 1.0 and 3.0 μg/rat) and that to L-arginine plus morphine (5.0 mg/kg) [$F(3,56)=0.5$, $P>0.05$]. None of the drugs induced locomotor activity, but the combination of morphine with L-arginine elicited an increase in locomotion.

Fig. 4A shows the effect of L-NAME on the morphine-induced conditioned place preference. Two-way ANOVA

indicates no significant interaction between the responses to L-NAME (0.3, 1.0 and 3.0 μg/rat) in the presence or absence of morphine (5.0 mg/kg) [$F(3,56)=0.3$, $P>0.05$]. Analysis also shows no response for L-NAME alone.

Fig. 4B shows the response to L-NAME and/or morphine for locomotor activity. Two-way ANOVA shows no significant difference between the effect of L-NAME with or without morphine [$F(3,56)=1.2$, $P>0.05$]. The data show that none of the drugs altered locomotion.

Fig. 5A shows the effect of L-NAME on the conditioned place preference induced by the combination of morphine with L-arginine. One-way ANOVA shows a significant difference between the response to morphine alone and that to morphine (5.0 mg/kg) plus L-arginine (1.0 μg/rat) in the presence or absence of L-NAME (0.3, 1.0 and 3.0 μg/rat) [$F(4,35)=22.3$, $P<0.0001$]. Further analysis indicates that L-arginine enhanced the morphine-induced conditioned place preference, and L-NAME preadministration reduced the response induced by the combination of L-arginine with morphine.

Fig. 5B shows the response to L-NAME for the locomotion elicited by the combination of L-arginine plus morphine. One-way ANOVA indicates that morphine plus L-arginine induced locomotion, which was reduced by L-NAME preadministration [$F(4,35)=7.1$, $P<0.001$].

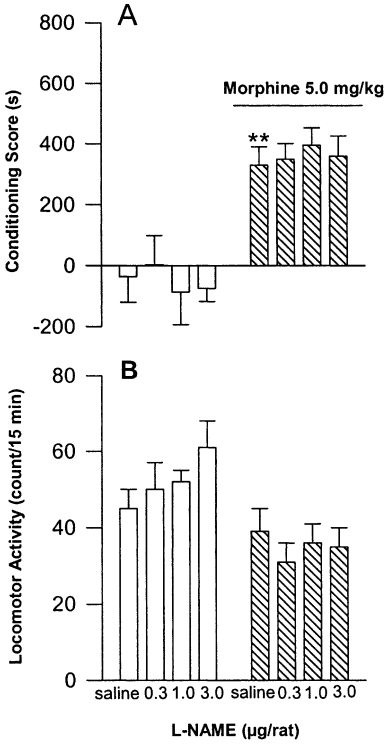

Fig. 4. Effect of L-NAME with or without morphine on conditioned place preference. Animals received saline (1.0 μl/rat, intra-central amygdala) or L-NAME (0.3, 1.0 and 3.0 μg/rat, intra-central amygdala) in the presence or absence of morphine (5.0 mg/kg, s.c.) during conditioning. The data are expressed as mean conditioning scores ± S.E.M. The conditioning score is defined as the time (in s) spent in the drug-paired place minus that spent in the saline-paired place (A). Locomotor activity was assessed as described in Materials and methods (B). ** $P<0.01$ different from the saline control group.

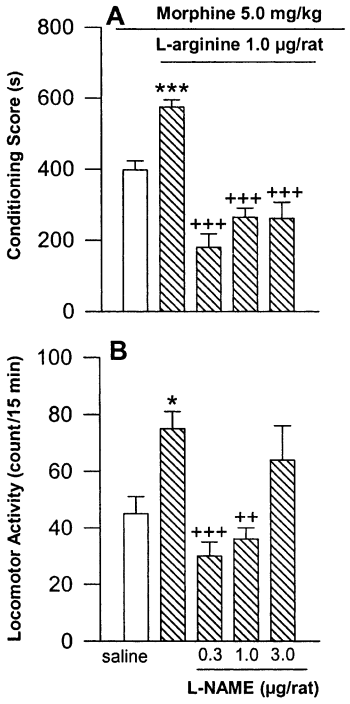

Fig. 5. Effect of L-NAME on the conditioned place preference-induced by the combination of morphine with L-arginine. Animals received L-NAME (0.3, 1.0 and 3.0 μg/rat, intra-central amygdala) 1 min before L-arginine (1.0 μg/rat, intra-central amygdala) injection and then they were injected with morphine (5.0 mg/kg, s.c.) during conditioning. The saline control group received saline (1.0 μl/rat, intra-central amygdala) 1 min before morphine (5.0 mg/kg, s.c.) injection during conditioning. The L-arginine control group received L-arginine (1.0 μg/rat, intra-central amygdala) 1 min before morphine (5.0 mg/kg, s.c.) administration during conditioning. The data are expressed as mean conditioning scores ± S.E.M. The conditioning score is defined as the time (in s) spent in the drug-paired place minus that spent in the saline-paired place (A). Locomotor activity was assessed as described in Materials and methods (B). * $P<0.05$, *** $P<0.001$ different from the saline control group. ++ $P<0.01$, +++ $P<0.001$ different from the L-arginine control group.

3.3. Effect of NO synthesis precursor and inhibitor on the expression of morphine-induced conditioned place preference

Fig. 6A shows the effect of L-arginine or L-NAME on the expression of morphine-induced conditioned place preference. L-arginine and L-NAME were administered on the test day, 1 min before conditioned place preference testing in a morphine-free state (as described in Materials and methods). One-way ANOVA shows that L-arginine (0.3, 1.0 and 3.0 μg/rat), but not L-NAME (0.3, 1.0 and 3.0 μg/rat), increased the expression of the morphine-

induced conditioned place preference [$F(6,49)=4.3$, $P<0.01$].

Fig. 6B shows the effect of L-arginine or L-NAME on locomotor activity in morphine-conditioned animals. One-way ANOVA indicates that the administration of only the lower dose of L-arginine (0.3 μg/rat), before testing of morphine-induced conditioned place preference, caused an increase in locomotion [one-way ANOVA; $F(6,49)=8.4$, $P<0.001$].

Fig. 7A shows the effect of L-arginine in the presence or absence of L-NAME on the expression of morphine-induced conditioned place preference. One-way ANOVA shows that L-arginine potentiated the morphine conditioning, and L-NAME (0.3, 1.0 and 3.0 μg/rat) decreased the response induced by morphine plus L-arginine (0.3 μg/rat) [$F(4,35)=7.5$, $P<0.001$].

Fig. 7B shows the response to L-arginine, with or without L-NAME on locomotor activity, on the testing day, of morphine-conditioned animals. Analysis indicates that the administration of L-arginine pretesting caused an increase in locomotion which was reduced by one dose of L-NAME (1.0 μg/rat) [one-way ANOVA; $F(4,35)=6.8$, $P<0.001$].

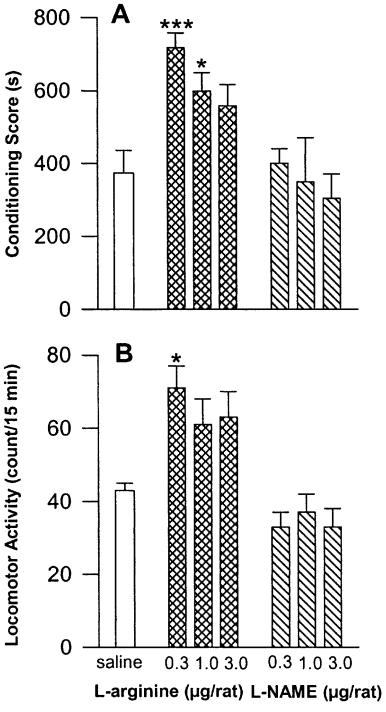

Fig. 6. Effects of L-arginine or L-NAME on the expression of morphine-induced conditioned place preference. Animals received morphine (5.0 mg/kg, s.c.) or saline (1.0 ml/kg, s.c.) in a 3-day schedule of conditioning. On the testing day, saline (1.0 μl/rat, intra-central amygdala), L-arginine (0.3, 1.0 and 3.0 μg/rat, intra-central amygdala) or L-NAME (0.3, 1.0 and 3.0 μg/rat, intra-central amygdala) was injected 1 min before testing. The data are expressed as mean conditioning scores ± S.E.M. The conditioning score is defined as the time (in s) spent in the drug-paired place minus that spent in the saline-paired place (A). Locomotor activity was assessed as described in Materials and methods (B). * $P<0.05$, *** $P<0.001$ different from the saline control group.

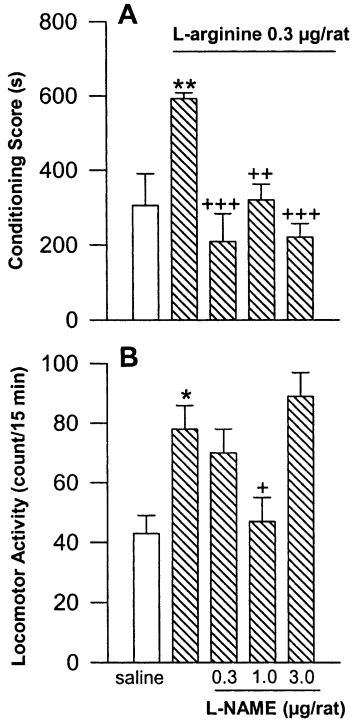

Fig. 7. Effect of L-arginine with or without L-NAME on the expression of morphine-induced conditioned place preference. Animals received morphine (5.0 mg/kg, s.c.) or saline (1.0 ml/kg, s.c.) in a 3-day schedule of conditioning. On the testing day, L-NAME (0.3, 1.0 and 3.0 μg/rat, intra-central amygdala) was administered 1 min before L-arginine (0.3 μg/rat, intra-central amygdala) injection. Saline control group received saline (1.0 μl/rat, intra-central amygdala) before testing. The L-arginine control group received L-arginine (0.3 μg/rat, intra-central amygdala) before testing. The data are expressed as mean conditioning scores ± S.E.M. The conditioning score is defined as the time (in s) spent in the drug-paired place minus that spent in the saline-paired place (A). Locomotor activity was assessed as described in Materials and methods (B). * $P<0.05$, ** $P<0.01$ different from the saline control group. + $P<0.05$, ++ $P<0.01$, +++ $P<0.001$ different from the L-arginine control group.

4. Discussion

The present study concerned the effects of intra-central amygdala injections of L-arginine, a precursor of nitric oxide (NO), and L-NAME, a NO synthase (NOS) inhibitor, on the acquisition and expression of morphine-induced conditioned place preference. Our data indicate that morphine induced a significant conditioned place preference in an unbiased conditioned place preference setup, which is in agreement with results of others in this respect (Shippenberg et al., 1996). The results showed no significant effect on locomotion as compared with the saline control group. Our previous results had shown that the dose of 10.0 mg/kg of morphine induces significant locomotor activity (Zarrindast and Zarghi, 1992). There is further evidence (Sukhotina, 2001; Lu et al., 2002)

indicating that the conditioned stimulus is a critical determinant of the form of conditioned response in a morphine locomotor conditioning setup. Based on the Bevins and Bardo (1998) report, a stimulus element, which reliably paired with morphine, evoked morphine-induced conditioned hypoactivity at 2 to less than 8 mg/kg in rats.

The present study indicates that intra-central amygdala administration of single doses of L-arginine, a precursor of NO, or L-NAME, a NOS inhibitor, did not elicit any response regarding conditioning. L-NAME has been shown to be ineffective as to morphine reinforcement properties (Zarrindast et al., 2002). Furthermore, our data indicate that co-administration of L-arginine, but not L-NAME, with morphine during conditioning, increased morphine-induced conditioned place preference and also induced an increase in locomotion. These responses, which are induced by morphine plus L-arginine, were reduced by the administration of L-NAME, an inhibitor of NO formation, which competes with the precursor (L-arginine) for NOS (Bozarth et al., 1993). One explanation may be that the increase in the NO levels enhances the morphine conditioning. This possibility can be supported by the data indicating that NO may play a role in morphine tolerance and dependence (Dambisya and Lee, 1996; Majeed et al., 1994; Machelska et al., 1997; Lue et al., 1999) and also in the rewarding properties of opiates (Kivastik et al., 1996; Itzhak et al., 1998). Since the central amygdala is linked with the motor system (LeDoux, 1993), an influence of locomotion on the L-arginine-induced potentiation of morphine conditioning cannot be excluded.

The present study also considered the effects of L-arginine and L-NAME on the expression of morphine-induced conditioned place preference. The administration of L-arginine, but not of L-NAME pretesting, increased the expression of morphine-induced conditioned place preference, but only the lower dose of L-arginine (0.3 μg/rat) increased locomotion. Intra-central amygdala administration of L-NAME 1 min before the injection of L-arginine reduced the enhancement induced by L-arginine. These effects indicate that NO production may be implicated in the expression of morphine-induced conditioned place preference. Although the exact mechanism of this process is not clear, it may further support the involvement of NO in morphine rewarding. In support of our results, there are reports indicating that the amygdala is involved in stimulus-reward associations (Hiroi and White, 1991). On the other hand, extensive evidence implicates the central amygdala in aversive properties of opiates (Ghallaghar et al., 1990; Gracy et al., 2001). Our results may indicate that the central amygdala is also involved in the positive reinforcement properties of opiates, a process that needs to be clarified.

It has been shown that the dopaminergic system is involved in rewarding (Robinson and Becker, 1986; Bardo, 1998; McBride et al., 1999). Dopamine terminals in the amygdala are distributed primarily in the central nucleus of the amygdala (Ben-Ari et al., 1975). It has been postulated that the dopaminergic behavior of cocaine, and also cocaine-induced reverse tolerance and conditioned place preference, may be mediated partially via activation of the NO system (Kim and Park, 1995). Moreover, evidence suggests that NO is a retrograde transmitter that signals to presynaptic neurons, causing an increase in the release of dopamine (Pudiak and Bozarth, 1993). Therefore, NO may be a neuronal messenger mediating the release of dopamine in the central nucleus of the rat amygdala, the mechanism by which the enhanced morphine-induced conditioned place preference in the pre-experiments could be explained.

As conditioned place preference is a learning test in that, during testing, animals must remember the place and cues associated with drug administration (Olmstead and Franklin, 1997a), the role of NO system in mediation of this type of learning should be considered.

Our results showed that the central amygdala may be involved in the acquisition and expression of morphine-induced conditioned place preference and further studies may be required to elucidate the exact role of NO and the central amygdala in the reward effects of drugs.

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